REVIEW

Molecular evolution of the growth hormone-releasing hormone/pituitary adenylate cyclase-activating polypeptide gene family. Functional implication in the regulation of growth hormone secretion

M Montero, L Yon, S Kikuyama[1], S Dufour[2] and H Vaudry

European Institute for Peptide Research (IFRMP 23), Laboratory of Cellular and Molecular Neuroendocrinology, INSERM U413, UA CNRS, University of Rouen, 76821 Mont-Saint-Aignan, France

[1]Laboratory of Endocrinology, Department of Biology, School of Education, Waseda University, Nishi-Waseda 1-6-1, Tokyo 169–8050, Japan

[2]Laboratory of General and Comparative Physiology, CNRS UMR 8572, National Museum of Natural History, 75005 Paris, France

(Requests for offprints should be addressed to H Vaudry)

ABSTRACT

Growth hormone-releasing hormone (GHRH) and pituitary adenylate cyclase-activating polypeptide (PACAP) belong to the same superfamily of regulatory neuropeptides and have both been characterized on the basis of their hypophysiotropic activities. This review describes the molecular evolution of the GHRH/PACAP gene family from urochordates to mammals and presents the hypothesis that the respective roles of GHRH and PACAP in the control of GH secretion are totally inverted in phylogenetically distant groups of vertebrates. In mammals, GHRH and PACAP originate from distinct precursors whereas, in all submammalian taxa investigated so far, including birds, amphibians and fish, a single precursor encompasses a GHRH-like peptide and PACAP. In mammals, GHRH-containing neurons are confined to the infundibular and dorsomedial nuclei of the hypothalamus while PACAP-producing neurons are widely distributed in hypothalamic and extrahypothalamic areas. In fish, both GHRH- and PACAP-immunoreactive neurons are restricted to the diencephalon and directly innervate the adenohypophysis. In mammals and birds, GHRH plays a predominant role in the control of GH secretion. In amphibians, both GHRH and PACAP are potent stimulators of GH release. In fish, PACAP strongly activates GH release whereas GHRH has little or no effect on GH secretion. The GHRH/PACAP family of peptides thus provides a unique model in which to investigate the structural and functional facets of evolution.

Journal of Molecular Endocrinology (2000) **25,** 157–168

INTRODUCTION

Growth hormone-releasing hormone (GHRH) was initially characterized from a human pancreatic tumor on the basis of its ability to stimulate growth hormone (GH) secretion from adenohypophyseal cells (Guillemin *et al.* 1982, Rivier *et al.* 1982). Pituitary adenylate cyclase-activating polypeptide (PACAP) was first identified from the ovine hypothalamus as a result of its capability of stimulating cAMP formation in rat anterior pituitary cells (Miyata *et al.* 1989). Soon after the discovery of PACAP, it became apparent that these two hypophysiotropic neuropeptides exhibit a

0952–5041/00/025–157

	1 5 10 15 20 25 30 35 40 45	
PACAP38	H S D G I F T D S Y S R Y R K Q M A V K K Y L A A V L G K R Y K Q R V K N K a	(1, 2)
PACAP 27	- a	(1, 2)
VIP	- - - A V - - - N - T - L - - - - - - - - - - N S I - N a	(3)
GHRH	Y A - A - - - N - - - K V L G - L S A R - L - Q D I M S R Q Q G E S N Q E R G A R A R L a	(4, 5, 6)
Secretin	- - - - T - - S E L - - L - E G A R L Q R L - Q G L V a	(7)
Glucagon	- - Q - T - - S D - - K - L D S R R A Q D F V Q W L M N T	(8)
GLP-2	- A - - S - S D E M N T I L D N L - A R D F I N W L I Q T K I T D	(8)
PRP	D V A H - - L N E A - R K V L D - L S A G - H - Q S L V A a	(1, 2)
PHM	- A - - V - - S D F - K L L G - L S A - - - - E S L M a	(3)
GIP	Y A E - T - I S D - - I A M D K I H Q Q D F V N W L - A Q - G - K N D W K H N I T Q	(9)

FIGURE 1. Primary structures of the different members of the GHRH superfamily in human. (1) Kimura *et al.* 1990, (2) Ohkubo *et al.* 1992, (3) Itoh *et al.* 1983, (4) Rivier *et al.* 1982, (5) Guillemin *et al.* 1982, (6) Ling *et al.* 1984, (7) Carlquist *et al.* 1985, (8) Bell *et al.* 1983, (9) Moody *et al.* 1984. Abbreviations as in the text; a, amidated; –, amino acid identical to that of PACAP38.

	1 5 10 15 20 25 30 35 40 45	
GHRH		
human	Y A D A I F T N S Y R K V L G Q L S A R K L L Q D I M S R Q Q G E S N Q E R G A R A R L a	(1-3)
pig	- R - - - Q - - - V - - a	(4)
cattle, goat	- N - - - - - R - - - Q - - K V - - a	(5, 6)
sheep	- - - - - - - - - - - - I - - - - - - - - - - - - - - N - - - - - R - - - Q - - K V - - a	(6)
hamster	- - - - - - - S - R - - - Q - P - S V R L	(7)
rat	H - - - - - - S - - - R I - - - - Y - - - - - H E - - N - - - - - R - - - Q R S - F N	(8, 9)
mouse	H V - - - - - T N - - - L - S - - Y - - - V I - - - - N K - - - - R I - - Q R - - L S	(10, 11)
chicken	H - - G - - S K A - - - L - - - - - - - N Y - H S L - A K R V - G A S S - L - D E - E P L S	(12)
frog	H - - D L L N K A - - N L - - - - - - - - Y - H T L - A K H L - A V S S S L E D D S E P L S	(13)
carp	H - - G M - N K A - - - A - - - - - - - - Y - H T L - A K R V - G G S M I E D D N E P L S	(14)
salmon	H - - G M - N K A - - - A - - - - - - - R - - H S L - A K R V - G G S T M E D D T E P L S	(15)
catfish	H - - G L L D R A L - D I - V - - - - - - Y - H S L T A V R V - - E E E D E E D S E P L S	(16)
sea squirt 1	H S - G - - - K D - - - Y - - - - R - Q - F - - W L -	(17)
sea squirt 2	H S - G - - - S D - - R Y - - - - - - Q - F - - W L -	(17)
PACAP		
mammals (human, sheep, rat, mouse)	H S D G I F T D S Y S R Y R K Q M A V K K Y L A A V L G K R Y K Q R V K N K a	(18-25)
chicken	- I -	(12)
lizard	- -........ ?	(26)
frog	- I - - - a	(13, 27)
catfish	- R - - R - - F R - - a	(16)
salmon	- R - - Y R - - a	(15)
stargazer	- - - - - - - - - - - - - - - - - - - Q - - - - - - - - R - - R - - - R - - a	(28)
stingray	- P K - R - S G R R V F Y	(29)
sea squirt 1	- - - - - - - - - - - - - - N - - - - - - - - - - - - a	(17)
sea squirt 2	- - - - - - - - - - - - - - N - - - - - - - I N - L - a	(17)

FIGURE 2. Comparison of the primary structures of GHRH and PACAP in different species of vertebrates and urochordates. (1) Rivier *et al.* 1982, (2) Guillemin *et al.* 1982, (3) Ling *et al.* 1984, (4) Böhlen *et al.* 1983, (5) Esch *et al.* 1983, (6) Brazeau *et al.* 1984, (7) Ono *et al.* 1994, (8) Spiess *et al.* 1983, (9) Böhlen *et al.* 1984, (10) Frohman *et al.* 1989, (11) Suhr *et al.* 1989, (12) McRory *et al.* 1997, (13) Alexandre *et al.* 2000, (14) Vaughan *et al.* 1992, (15) Parker *et al.* 1993, (16) McRory *et al.* 1995, (17) McRory & Sherwood 1997, (18) Miyata *et al.* 1989, (19) Kimura *et al.* 1990, (20) Miyata *et al.* 1990, (21) Ogi *et al.* 1990, (22) Hosoya *et al.* 1992, (23) Ohkubo *et al.* 1992, (24) Okazaki *et al.* 1995, (25) Yamamoto *et al.* 1998, (26) Pohl & Wank 1998, (27) Chartrel *et al.* 1991, (28) Matsuda *et al.* 1997*a*, (29) Matsuda *et al.* 1998. a, amidated; –, amino acid identical to that of human GHRH or mammalian PACAP.

number of structural and functional similarities. (i) They stand among the largest hypothalamic neurohormones discovered so far. (ii) They possess considerable sequence similarity so that it is generally accepted that they arose by genomic duplication of the exon encoding a common ancestor. (iii) They both appeared very early during evolution prior to the emergence of vertebrates. (iv) They are widely distributed in peripheral organs, notably in the gut, pancreas, ovary and testis. (v) Their biological effects are mediated through 7-transmembrane domain receptors positively coupled to adenylyl cyclase. Besides these similarities, GHRH and PACAP display one major

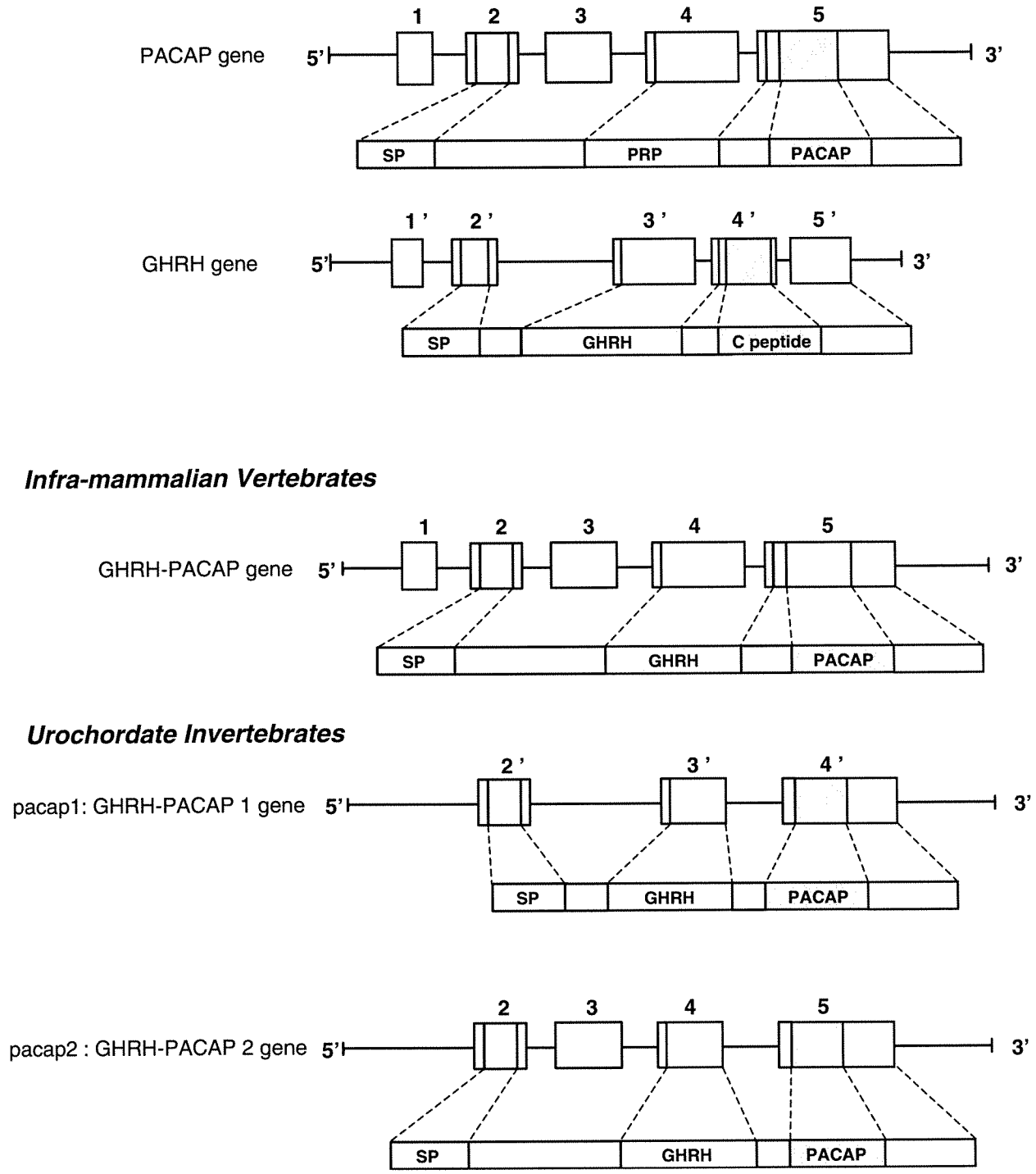

FIGURE 3. Organization of the GHRH, PACAP and GHRH/PACAP genes of vertebrates and invertebrates. Exon numbering follows that used for the mammalian genes.

difference: the sequence of GHRH has been poorly conserved, even in closely related species, while the sequence of PACAP has been extremely well preserved during evolution.

Examination of the amino acid sequences of GHRH and PACAP clearly indicates that these two neuropeptides belong to a superfamily of regulatory peptides which encompasses glucagon, secretin, vasoactive intestinal polypeptide (VIP), peptide histidine methionine (PHM) or peptide histidine isoleucine (PHI), gastric inhibitory peptide (GIP), and glucagon-like peptides (GLPs) (Fig. 1) (Campbell & Scanes 1992, Kieffer & Habener 1999). Molecular characterization of the GHRH and PACAP precursors in several representative species of urochordates and vertebrates, together with studies on their physiological implication in the control of pituitary hormone secretion, have provided important information pertaining to the evolutionary aspects of their structures and functions. The aim of the present review is to summarize the current knowledge regarding the phylogenetic origin and the evolution of the respective roles of GHRH and PACAP in the regulation of GH secretion.

MOLECULAR DIVERSITY OF GHRH AND PACAP

Evolution of the primary structures of GHRH and PACAP

The sequence of GHRH was initially determined from a tumor which had caused acromegaly (Guillemin *et al.* 1982, Rivier *et al.* 1982). Subsequently, the primary structure of the peptide has been elucidated in a number of species, either directly determined after peptide purification or deduced from a cloned cDNA. In contrast to most hypophysiotropic neurohormones whose sequences have been generally well conserved during evolution, the primary structure of GHRH is highly variable (Fig. 2). In particular, rat and mouse GHRHs only exhibit 67% sequence identity, which is by far the lowest degree of similarity for regulatory neuropeptides observed between these two rodent species.

Two molecular forms of PACAP with 38 (PACAP38) and 27 (PACAP27) amino acids were originally isolated from the sheep hypothalamus (Miyata *et al.* 1989, 1990). The sequence of PACAP has been remarkably well preserved from the sea squirt (an urochordate invertebrate) to the human (Fig. 2). In particular, the sequence of PACAP38 is identical in all mammalian species studied so far. In other vertebrates, strong evolutionary pressure has acted to preserve the sequence of the N-terminal domain which corresponds to the biologically active region of the molecule (Gonzalez *et al.* 1998). It is noteworthy that the sequences of the two tunicate PACAP27 variants exhibit only one and four amino acid substitutions as compared with their mammalian ortholog (Miyata *et al.* 1990, McRory & Sherwood 1997). Such a high degree of peptide sequence conservation between two taxa which have diverged from a common ancestor some 600 million years ago (McRory & Sherwood 1997) indicates that PACAP fulfils important biological functions.

Evolution of the structure of the GHRH and PACAP precursors

In mammals, the GHRH and PACAP precursors are encoded by two distinct genes (Mayo *et al.* 1985*a*, Hosoya *et al.* 1992). Each of these precursors encompasses two related peptides: the GHRH precursor comprises GHRH and a C-terminal flanking peptide called C-peptide, while the PACAP precursor comprises PACAP and an N-terminal flanking peptide called PACAP-related peptide or PRP (Fig. 3). Comparison of the sequences of these peptides reveals a higher degree of similarity between PRP and GHRH than between PRP and PACAP (45% and 21% respectively in human).

In contrast, in all submammalian species investigated so far, a GHRH-like peptide and PACAP are located on the same precursor (Fig. 3). To date, the cDNA encoding the GHRH/PACAP precursor has been characterized in the chicken *Gallus domesticus* (McRory *et al.* 1997), the frog *Rana ridibunda* (Alexandre *et al.* 2000), the catfish *Clarius macrocephalus* (McRory *et al.* 1995) and the salmon *Oncorhynchus nerka* (Parker *et al.* 1993). In birds, amphibians and fish, alternative splicing of the primary transcript can generate either a long mRNA encompassing both the GHRH and PACAP sequences, or a short mRNA lacking the GHRH sequence (McRory *et al.* 1997, Parker *et al.* 1997, Alexandre *et al.* 2000). Two cDNAs encoding GHRH/PACAP precursors have also been cloned in an urochordate, the sea squirt *Chelyosoma productum* (McRory & Sherwood 1997). The organization of the GHRH/PACAP genes in urochordates exhibits strong similarities with those of vertebrates (Fig. 3). Sequence analysis of the two GHRH/PACAP precursors of the sea squirt reveals a higher degree of similarity between the two GHRH sequences (89%) and the two PACAP sequences (89%) than between the GHRH and PACAP sequences within each precursor (41% for the GHRH/PACAP1 precursor and 48% for the

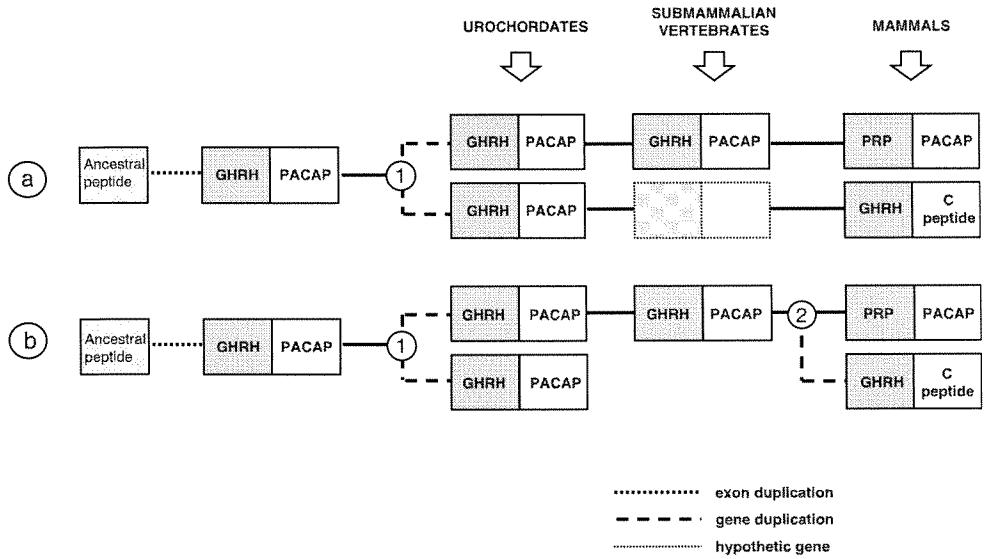

FIGURE 4. Proposed evolutionary history of the GHRH/PACAP precursor family. In both hypotheses a and b, it is assumed that exon duplication, leading to the appearance of the GHRH/C peptide and PRP/PACAP sequences, occurred before gene duplication. Hypothesis a: a single duplication would have occurred before the emergence of urochordates. Hypothesis b: two duplications would have occurred independently in the urochordates and in a common ancestor of mammals.

GHRH/PACAP2 precursor), suggesting that exon duplication generating GHRH and PACAP occurred before duplication of the ancestral gene (Fig. 4).

Since only one GHRH/PACAP cDNA has been characterized to date in teleosts (Parker *et al.* 1993, McRory *et al.* 1995), amphibians (Alexandre *et al.* 2000) and birds (McRory *et al.* 1997) while two distinct cDNAs encoding either GHRH or PACAP have been cloned in rat (Mayo *et al.* 1985*b*, Ogi *et al.* 1990), mouse (Frohman *et al.* 1989, Suhr *et al.* 1989, Okazaki *et al.* 1995) and human (Gübler *et al.* 1983, Mayo *et al.* 1983, 1985*a*, Kimura *et al.* 1990, Hosoya *et al.* 1992, Ohkubo *et al.* 1992), it has been proposed that a gene duplication event occurred just before the emergence of mammals (Arimura 1998, Hoyle 1998, Vaudry *et al.* 2000). However, several observations suggest that a second gene encoding a GHRH-like peptide may well exist in non-mammalian species (Fig. 4). In particular, two GHRH/PACAP genes are present in urochordates, suggesting that duplication of the ancestral gene may have occurred very early during evolution (McRory & Sherwood 1997). In fact, the organization of the GHRH/PACAP1 gene of the sea squirt exhibits much similarity with that of the mammalian GHRH precursor gene, while the structure of the GHRH/PACAP2 gene of the sea squirt resembles that of the mammalian PACAP precursor gene (Fig. 4), suggesting that the two urochordate genes may be orthologous to the two mammalian genes. In that case, the existence of two GHRH/PACAP precursors should also occur in submammalian vertebrate species. Indeed, Southern blot analysis of salmon genomic DNA has revealed the existence of at least two bands hybridizing with a probe including the GHRH/PACAP coding region (Parker *et al.* 1993) and two variants of GHRH/PACAP cDNAs have recently been cloned in the goldfish (Leung *et al.* 1999), confirming the existence of more than one GHRH/PACAP gene in teleosts.

EVOLUTION OF THE DISTRIBUTION OF GHRH AND PACAP NEURONS IN THE BRAIN

In mammals, GHRH-containing neurons are strictly located in the arcuate and the dorsomedial nuclei of the hypothalamus (Mayo *et al.* 1995), whereas PACAP-containing perikarya are widely distributed, not only in the hypothalamus, but also in the thalamus, septum, cortex, amygdala, hippocampal area, cerebellum and pons (Arimura *et al.* 1991, Koves *et al.* 1991, 1994, Ghatei *et al.* 1993, Legradi *et al.* 1994, Nielsen *et al.* 1998) (Fig. 5a).

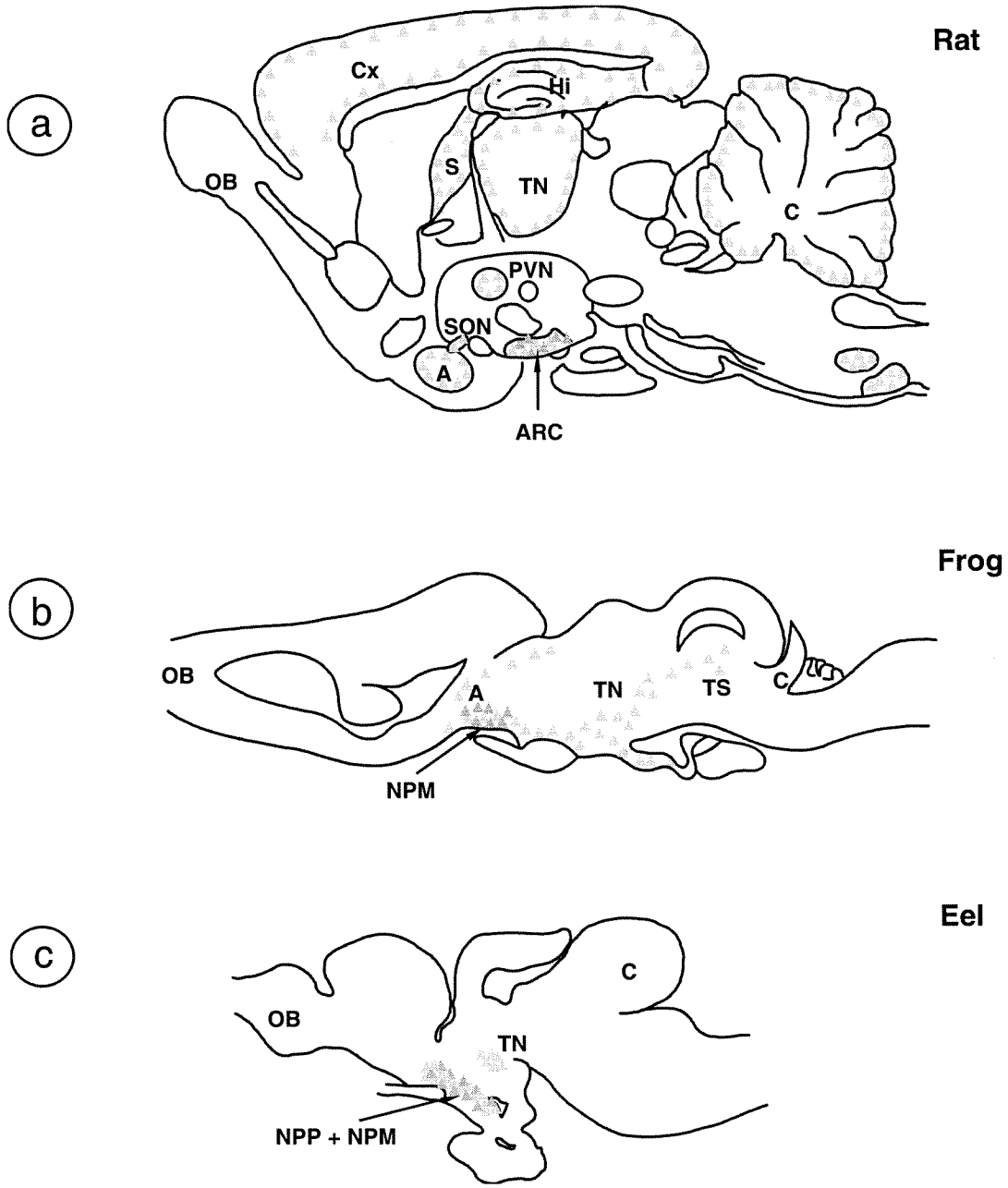

FIGURE 5. Schematic parasagittal sections through (a) rat, (b) frog and (c) eel brain depicting the distribution of GHRH and PACAP perikarya. Red triangles, GHRH-immunoreactive cell bodies; green triangles, PACAP-immunoreactive cell bodies. A, amygdala; ARC, arcuate nucleus; C, cerebellum; Cx, cortex; Hi, hippocampus; NPM, magnocellular preoptic nucleus; NPP, posterior preoptic nucleus; OB, olfactory bulb; PVN, paraventricular nucleus; S, septum; SON, supraoptic nucleus; TN, thalamic nucleus; TS, torus semicircularis. Adapted from Gonzalez *et al.* 1998, Montero *et al.* 1998 and Yon *et al.* 1992.

In birds, the distribution of GHRH neurons has never been reported while the location of PACAP-containing neurons has been studied in the chicken forebrain (Peeters *et al.* 1998*a*). PACAP-immunoreactive cell bodies are particularly abundant in the ventral aspect of the supraoptic nucleus, as well as in the magnocellular subdivisions of the preoptic and paraventricular nuclei (Peeters *et al.* 1998*a*).

In amphibians, unfortunately, the distribution of GHRH neurons has been exclusively investigated in the hypothalamo–hypophyseal system, so that the presence of GHRH-immunoreactive cell bodies has only been described in the magnocellular part of the preoptic nucleus (Marivoet *et al.* 1988). The existence of PACAP-positive perikarya has been reported in the frog hypothalamus as well as in extrahypothalamic areas including the thalamus, amygdala and torus semicircularis (Yon *et al.* 1992) (Fig. 5b). Since, in frog, a GHRH-like peptide and PACAP are encoded by the same gene (Alexandre *et al.* 2000), it is conceivable that GHRH-like immunoreactivity could have a more widespread distribution than initially described (Marivoet *et al.* 1988). Thus, the location of GHRH-containing neurons in the amphibian brain outside the hypothalamic region deserves to be investigated.

In fish, GHRH-immunoreactive neurons are primarily located in the magnocellular and parvocellular portions of the preoptic nucleus; a few GHRH-positive neurons are also found in the rostral part of the nucleus lateralis tuberis (Marivoet *et al.* 1988, Olivereau *et al.* 1990). Similarly, PACAP-positive cell bodies are found in the preoptic nucleus and in the nucleus lateralis tuberis (Matsuda *et al.* 1997*b*, Montero *et al.* 1998, Yoshida *et al.* 1999) (Fig. 5c). The expression of PACAP mRNA in the preoptic area has recently been confirmed by *in situ* hybridization (Satoh *et al.* 1999). The co-localization of GHRH and PACAP in the same regions of the fish diencephalon is consistent with the occurrence of a GHRH-like peptide and PACAP within the same precursor (Parker *et al.* 1993, McRory *et al.* 1995). Interestingly, in teleost fish, fibers immunoreactive for GHRH and PACAP directly innervate the pars distalis (Marivoet *et al.* 1988, Olivereau *et al.* 1990, Matsuda *et al.* 1997*b*, Montero *et al.* 1998), suggesting that both GHRH and PACAP may be involved in the control of pituitary hormone secretion.

EVOLUTION OF THE REGULATION OF GH SECRETION BY GHRH AND PACAP

In mammals, GH secretion is primarily under the stimulatory control of GHRH and the inhibitory influence of somatostatin (Bertherat *et al.* 1995). Studies aimed at investigating the effect of PACAP on GH secretion have led to controversial results. Specifically, some data suggest that PACAP stimulates GH release in rat (Goth *et al.* 1992, Hart *et al.* 1992, Jarry *et al.* 1992, Leonhardt *et al.* 1992, Nagy *et al.* 1993, Wei *et al.* 1993, Velkeniers *et al.* 1994), sheep (Sawangjaroen *et al.* 1997) and cattle (Hashizume *et al.* 1994), whereas other reports indicate that PACAP has no effect on GH secretion (Miyata *et al.* 1989, Culler & Paschall 1991, Jarry *et al.* 1992, Sawangjaroen & Curlewis 1994). In humans, intravenous administration of PACAP does not modify plasma GH levels (Chiodera *et al.* 1996) and PACAP is less potent than GHRH in stimulating GH release from somatotropic adenoma cells in primary culture (Adams *et al.* 1994). In birds, PACAP has been found to induce a dose-dependent stimulation of cAMP formation by chicken pituitary cells. However, PACAP causes only a modest increase of GH release as compared with the robust stimulatory effect of human GHRH (Peeters *et al.* 1998*b*). Taken together, these data indicate that PACAP probably plays a minor role in the control of GH secretion in mammals and birds (Table 1).

TABLE 1. Relative implication of GHRH and PACAP in the control of GH secretion in vertebrates

	GHRH	PACAP
Taxa		
Mammals	+++	+/0
Birds	+++	+
Reptiles	?	?
Amphibians	+++	+++
Teleosts	+/0	+++

In amphibians, frog (f) PACAP stimulates GH secretion in the European green frog *Rana ridibunda* (Martinez-Fuentes *et al.* 1994) and the bullfrog *Rana catesbeiana* (S Kikuyama, unpublished data). Frog PACAP stimulates adenylyl cyclase activity in frog pituitary slices (Yon *et al.* 1993) and increases cytosolic calcium concentration in cultured frog somatotrope cells (Gracia-Navarro *et al.* 1992). At least two types of PACAP receptors, i.e. type I receptors (PACAP-specific receptors) and type II receptors (PACAP-VIP receptors) are expressed in the distal lobe of frog and toad (Alexandre *et al.* 1999, Hu *et al.* 2000). Concurrently, it has been shown that human (h) GHRH stimulates GH secretion from bullfrog pituitary cells, and that the hGHRH-induced GH release is inhibited by somatostatin in a dose-dependent manner (Jeandel *et al.* 1998). In addition, we have recently found that fGHRH-like peptide and fPACAP are equipotent in stimulating GH secretion from cultured frog adenohypophyseal cells (S Kikuyama, unpublished data). These observations suggest that, in amphibians, GHRH and PACAP (in addition to

somatostatin) are involved in the neuroendocrine control of somatotrope cell activity (Table 1).

In fish, GHRH has been reported to exert a modest stimulatory effect in the trout *Oncorhynchus mykiss* (Luo *et al.* 1990, Blaise *et al.* 1995), the carp *Cyprinus carpio* (Vaughan *et al.* 1992) and the tilapia hybrid *Oreochromis niloticus* × *Oreochromis aureus* (Melamed *et al.* 1995) both *in vitro* and *in vivo*. In the salmon *Oncorhynchus nerka*, native GHRH produces only a weak stimulation of GH release from pituitary cells (Parker *et al.* 1997). In a primitive teleost, the European eel *Anguilla anguilla*, hGHRH is totally devoid of an effect on GH secretion (Montero *et al.* 1998). In contrast, both PACAP27 and PACAP38 provoke a robust stimulation of GH release from goldfish *Carassius auratus* (Wong *et al.* 1998) and eel pituitary cells (Montero *et al.* 1998). These observations strongly suggest that, in fish, PACAP rather than GHRH plays a physiological role in the control of GH secretion (Table 1). It should be noticed, however, that in the goldfish both type I PACAP receptor and a GHRH receptor are expressed in the pituitary (Chan *et al.* 1998, Wong *et al.* 1998), indicating that not only PACAP but also GHRH may exert some kind of hypophysiotropic activity. In addition, it has previously been shown that, in birds, amphibians and fish, several regulatory neuropeptides such as gonadotropin-releasing hormone (Marchant *et al.* 1989, Lin *et al.* 1993, Melamed *et al.* 1995), neuropeptide Y (Peng *et al.* 1990), thyrotropin-releasing hormone (Harvey *et al.* 1978, Gracia-Navarro *et al.* 1991, Trudeau *et al.* 1992), cholecystokinin (Himick *et al.* 1993), bombesin (Himick & Peter 1995) and corticotropin-releasing hormone (Rousseau *et al.* 1999) are involved in the control of somatotrope cell activity.

CONCLUSIONS

Since the identification of the sequence of secretin (Mutt *et al.* 1970), the characterization of multiple related peptides has provided insight into the molecular evolution of this large peptide superfamily. In the case of GHRH and PACAP, a series of exon and gene duplication events has led to the formation of a subfamily of neuropeptide precursors which exhibit the same overall organization. During vertebrate evolution, the primary structure of GHRH has markedly diverged while the sequence of PACAP has been strongly preserved. In submammalian vertebrates, GHRH and PACAP are arranged in tandem within the same precursor whereas, in mammals, the two peptides are generated from distinct precursors, suggesting that gene duplication may have occurred in a common ancestor during the transition period between birds and mammals (Fig. 4). Alternatively, submammalian vertebrates may possess two related GHRH/PACAP-encoding genes, one of which would remain to be characterized.

In all vertebrate species studied so far, the secretion of GH is under the dual control of inhibitory and stimulatory neuropeptides. While somatostatin acts as an inhibitor of GH release throughout the vertebrate phyllum, the respective roles of GHRH and PACAP as stimulatory factors of somatotrope cells have been radically inverted during evolution (Table 1). Recently, a novel hypothalamic neurohormone, termed ghrelin, has been characterized for its ability to stimulate GH secretion in mammals (Kojima *et al.* 1999). Identification of this novel neuropeptide in representative submammalian species and investigation of its possible GH-releasing activity will be necessary to understand the phylogenetic history of the control of GH secretion during vertebrate evolution.

ACKNOWLEDGEMENTS

This work was supported by grants from the Institut National de la Santé et de la Recherche Médicale (M M, L Y and H V), the Conseil Régional de Haute-Normandie (M M, L Y and H V), the Ministry of Education, Science and Culture of Japan (S K), the Centre National de la Recherche Scientifique (S D), the Conseil Supérieur de la Pêche (S D) and an INSERM-JSPS exchange program (S K and H V).

REFERENCES

Adams EF, Buchfelder M, Petersen B & Fahlbusch R 1994 Effect of pituitary adenylate cyclase-activating polypeptide on human somatotrophic tumours in cell culture. *Endocrine Journal* **2** 75–79.

Alexandre D, Anouar Y, Jégou S, Fournier A & Vaudry H 1999 A cloned frog vasoactive intestinal polypeptide/pituitary adenylate cyclase-activating polypeptide receptor exhibits pharmacological and tissue distribution characteristics of both VPAC1 and VPAC2 receptors in mammals. *Endocrinology* **140** 1285–1293.

Alexandre D, Vaudry H, Jégou S & Anouar Y 2000 Structure and distribution of the mRNAs encoding pituitary adenylate cyclase-activating polypeptide and growth hormone-releasing hormone-like peptide in the frog *Rana ridibunda*. *Journal of Comparative Neurology* **421** 234–246.

Arimura A 1998 Perspectives on pituitary adenylate cyclase-activating polypeptide (PACAP) in the neuroendocrine,

endocrine, and nervous systems. *Japanese Journal of Physiology* **48** 301–331.

Arimura A, Somogyvari-Vigh A, Miyata A, Mizuno K, Coy DH & Kitada C 1991 Tissue distribution of PACAP as determined by RIA: highly abundant in the rat brain and testes. *Endocrinology* **129** 2787–2789.

Bell GI, Sanchez-Pescador R, Laybourn PJ & Najarian RC 1983 Exon duplication and divergence in the human preproglucagon gene. *Nature* **304** 368–371.

Bertherat J, Bluet-Pajot MT & Epelbaum J 1995 Neuroendocrine regulation of growth hormone. *European Journal of Endocrinology* **132** 12–24.

Blaise O, Le Bail PY & Weil C 1995 Lack of gonadotropin-releasing hormone action on *in vivo* and *in vitro* growth hormone release in rainbow trout (*Oncorhynchus mykiss*). *Comparative Biochemistry and Physiology* **110C** 133–141.

Böhlen P, Esch F, Brazeau P, Ling N & Guillemin R 1983 Isolation and characterization of the porcine hypothalamic growth hormone-releasing factor. *Biochemical and Biophysical Research Communications* **116** 726–734.

Böhlen P, Wehrenberg WB, Esch F, Ling N, Brazeau P & Guillemin R 1984 Rat hypothalamic growth hormone-releasing factor: isolation, sequence analysis and total synthesis. *Biochemical and Biophysical Research Communications* **125** 1005–1012.

Brazeau P, Bohlen P, Esch F, Ling N, Wehrenberg WB & Guillemin R 1984 Growth hormone-releasing factor from ovine and caprine hypothalamus: isolation, sequence analysis and total synthesis. *Biochemical and Biophysical Research Communications* **125** 606–614.

Campbell RM & Scanes CG 1992 Evolution of the growth hormone-releasing factor (GRF) family of peptides. *Growth Regulation* **2** 175–191.

Carlquist M, Jörnvall H, Forssmann WG, Thulin L, Johansson C & Mutt V 1985 Human secretin is not identical to the porcine/bovine hormone. *IRCS Medical Science* **13** 217–218.

Chan KW, Yu KL, Rivier J & Chow BK 1998 Identification and characterization of a receptor from goldfish specific for a teleost growth hormone-releasing hormone-like peptide. *Neuroendocrinology* **68** 44–56.

Chartrel N, Tonon MC, Vaudry H & Conlon JM 1991 Primary structure of frog pituitary adenylate cyclase-activating polypeptide (PACAP) and effect of ovine PACAP on frog pituitary. *Endocrinology* **129** 3367–3371.

Chiodera P, Volpi R, Capretti L, Caffarri G, Magotti MG & Coiro V 1996 Effects of intravenously infused pituitary adenylate cyclase-activating polypeptide on adenohypophyseal hormone secretion in normal men. *Neuroendocrinology* **64** 242–246.

Culler MD & Paschall CS 1991 Pituitary adenylate cyclase-activating polypeptide (PACAP) potentiates the gonadotropin-releasing activity of luteinizing hormone-releasing hormone. *Endocrinology* **129** 2260–2262.

Esch F, Bohlen P, Ling N, Brazeau P & Guillemin R 1983 Isolation and characterization of the bovine hypothalamic growth hormone-releasing factor. *Biochemical and Biophysical Research Communications* **117** 772–779.

Frohman MA, Downs TR, Chomczynski P & Frohman LA 1989 Cloning and characterization of mouse growth hormone-releasing hormone (GRH) complementary DNA: increased GRH messenger RNA levels in the growth hormone-deficient lit/lit mouse. *Molecular Endocrinology* **3** 1529–1536.

Ghatei MA, Takahashi K, Suzuki Y, Gardiner J, Jones PM & Bloom SR 1993 Distribution, molecular characterization of pituitary adenylate cyclase-activating polypeptide and its precursor encoding messenger RNA in human and rat tissues. *Journal of Endocrinology* **136** 159–166.

Gonzalez BJ, Basille M, Vaudry D, Fournier A & Vaudry H 1998 Pituitary adenylate cyclase-activating polypeptide. *Annales d'Endocrinologie* **59** 364–405.

Goth MI, Lyons CE, Canny BJ & Thorner MO 1992 Pituitary adenylate cyclase activating polypeptide, growth hormone (GH)-releasing peptide and GH-releasing hormone stimulate GH release through distinct pituitary receptors. *Endocrinology* **130** 939–944.

Gracia-Navarro F, Castano JP, Malagon MM & Torronteras R 1991 Subcellular responsiveness of amphibian hormone cells after TSH-releasing hormone stimulation. *General and Comparative Endocrinology* **84** 94–103.

Gracia-Navarro F, Lamacz M, Tonon MC & Vaudry H 1992 Pituitary adenylate cyclase-activating polypeptide stimulates calcium mobilization in amphibian pituitary cells. *Endocrinology* **131** 1069–1074.

Gübler U, Monahan JJ, Lomedico PT, Bhatt RS, Collier KJ, Hoffman BJ, Böhlen P, Esch F, Ling N, Zeytin F, Brazeau P, Poonian MS & Gage LP 1983 Cloning and sequence analysis of cDNA for the precursor of human growth hormone-releasing factor, somatocrinin. *PNAS* **80** 4311–4314.

Guillemin R, Brazeau P, Böhlen P, Esch F, Ling N & Wehrenberg W 1982 Growth hormone-releasing factor from a human pancreatic tumor that caused acromegaly. *Science* **218** 585–587.

Hart GR, Gowing H & Burrin JM 1992 Effects of a novel hypothalamic peptide, pituitary adenylate cyclase-activating polypeptide, on pituitary hormone release in male rats. *Journal of Endocrinology* **134** 33–41.

Harvey S, Scanes CG, Chadwick A & Bolton NJ 1978 The effect of thyrotropin-releasing hormone (TRH) and somatostatin (GHRIH) on growth hormone and prolactin secretion *in vitro* and *in vivo* in the domestic fowl (*Gallus domesticus*). *Neuroendocrinology* **26** 249–260.

Hashizume T, Soliman EB & Kanematsu S 1994 Effects of pituitary adenylate cyclase-activating polypeptide (PACAP), prostaglandin E_2 (PGE_2) and growth hormone releasing factor (GRF) on the release of growth hormone from cultured bovine anterior pituitary cells *in vitro*. *Domestic Animal Endocrinology* **11** 331–337.

Himick BA & Peter RE 1995 Bombesin-like immunoreactivity in the forebrain and pituitary and regulation of anterior pituitary hormone release by bombesin in goldfish. *Neuroendocrinology* **61** 365–376.

Himick BA, Golosinski AA, Jonsson AC & Peter RE 1993 CCK/gastrin-like immunoreactivity in the goldfish pituitary: regulation of pituitary hormone secretion by CCK-like peptides *in vitro*. *General and Comparative Endocrinology* **92** 88–103.

Hosoya M, Kimura C, Ogi K, Ohkubo S, Miyamoto Y, Kugoh H, Shimizu M, Onda H, Oshimura M, Arimura A & Fujino M 1992 Structure of the human pituitary adenylate cyclase-activating polypeptide (PACAP) gene. *Biochimica et Biophysica Acta* **1129** 199–206.

Hoyle CHV 1998 Neuropeptide families: evolutionary perspectives. *Regulatory Peptides* **73** 1–33.

Hu Z, Lelievre V, Chao A, Zhou X & Waschek JA 2000 Characterization and messenger ribonucleic acid distribution of a cloned pituitary adenylate cyclase-activating polypeptide type I receptor in the frog *Xenopus laevis* brain. *Endocrinology* **141** 657–665.

Itoh N, Obata K, Yanaihara N & Okamoto H 1983 Human preprovasoactive intestinal polypeptide contains a novel PHI-27-like peptide, PHM-27. *Nature* **304** 547–549.

Jarry H, Leonhardt S, Schmidt WE, Creutzfeld W & Wuttke W 1992 Contrasting effects of pituitary adenylate cyclase-activating polypeptide (PACAP) on *in vivo* and *in vitro*

prolactin and growth hormone release in male rats. *Life Sciences* **51** 823–830.

Jeandel L, Okuno A, Kobayashi T, Kikuyama S, Tostivint H, Lihrmann I, Chartrel N, Conlon JM, Fournier A, Tonon MC & Vaudry H 1998 Effects of the two somatostatin variants somatostatin-14 and [Pro2, Met13]somatostatin-14 on receptor binding, adenylyl cyclase activity and growth hormone release from the frog pituitary. *Journal of Neuroendocrinology* **1998** 187–192.

Kieffer TJ & Habener JF 1999 The glucagon-like peptides. *Endocrine Reviews* **20** 876–913.

Kimura C, Ohkubo S, Ogi K, Hosoya M, Itoh Y, Onda H, Miyata A, Jiang L, Dahl RR & Stibbs HH 1990 A novel peptide which stimulates adenylate cyclase: molecular cloning and characterization of the ovine and human cDNAs. *Biochemical and Biophysical Research Communications* **166** 81–89.

Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H & Kangawa K 1999 Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* **402** 656–660.

Koves K, Arimura A, Gorcs TG & Somogyvari-Vigh 1991 Comparative distribution of immunoreactive pituitary adenylate cyclase activating polypeptide and vasoactive intestinal polypeptide in the rat forebrain. *Neuroendocrinology* **54** 159–169.

Koves K, Gorcs TJ, Kausz M & Arimura A 1994 Present status of knowledge about the distribution and colocalization of PACAP in the forebrain. *Acta Biologica Hungarica* **45** 297–321.

Legradi G, Shioda S & Arimura A 1994 Pituitary adenylate cyclase-activating polypeptide-like immunoreactivity in autonomic regulatory areas of the rat medulla oblongata. *Neuroscience Letters* **176** 193–196.

Leonhardt S, Jarry H, Kreipe A, Werstler K & Wuttke W 1992 Pituitary adenylate cyclase-activating polypeptide (PACAP) stimulates pituitary hormone release in male rats. *Neuroendocrinology Letters* **14** 319–328.

Leung MY, Tse LY, Yu KL, Chow BKC & Wong AOL 1999 Molecular cloning and tissue distribution of PACAP in the goldfish. In *Recent Progress in Molecular and Comparative Endocrinology*, pp 383–388. Eds HB Kwon, JMP Joss & S Ishii. Kwangju: Hormone Research Center, Chonnam National University.

Lin XW, Lin HR & Peter RE 1993 Growth hormone and gonadotropin secretion in the common carp (*Cyprinus carpio* L.): *in vitro* interactions of gonadotropin-releasing hormone, somatostatin, and the dopamine agonist apomorphine. *General and Comparative Endocrinology* **89** 62–71.

Ling N, Esch F, Böhlen P, Brazeau P, Wehrenberg WB & Guillemin R 1984 Isolation, primary structure, and synthesis of human hypothalamic somatocrinin: growth hormone-releasing factor. *PNAS* **81** 4302–4306.

Luo D, McKeown BA, Rivier J & Vale W 1990 *In vitro* responses of rainbow trout (*Oncorhynchus mykiss*) somatotrophs to carp growth hormone-releasing factor (GRF) and somatostatin. *General and Comparative Endocrinology* **80** 288–298.

McRory JE & Sherwood NM 1997 Two protochordate genes encode pituitary adenylate cyclase-activating polypeptide and related family members. *Endocrinology* **138** 2380–2390.

McRory JE, Parker DB, Ngamvongchon S & Sherwood NM 1995 Sequence and expression of cDNA for pituitary adenylate cyclase-activating polypeptide (PACAP) and growth hormone-releasing hormone (GHRH)-like peptide in catfish. *Molecular and Cellular Endocrinology* **108** 169–177.

McRory JE, Parker RL & Sherwood NM 1997 Expression and alternative processing of a chicken gene encoding both growth hormone-releasing hormone and pituitary adenylate cyclase-activating polypeptide. *DNA and Cell Biology* **16** 95–102.

Marchant TA, Chang JP, Nahorniak CS & Peter RE 1989 Evidence that gonadotropin-releasing hormone also functions as a growth hormone-releasing factor in the goldfish. *Endocrinology* **124** 2509–2518.

Marivoet S, Moons L & Vandesande F 1988 Localization of growth hormone-releasing factor-like immunoreactivity in the hypothalamo–hypophyseal system of the frog (*Rana temporaria*) and the sea bass (*Dicentrarchus labrax*). *General and Comparative Endocrinology* **72** 72–79.

Martinez-Fuentes AJ, Gonzalez de Aguilar JL, Lacuisse S, Kikuyama S, Vaudry H & Gracia-Navarro F 1994 Effect of frog pituitary adenylate cyclase-activating polypeptide (PACAP) on amphibian pituitary cells. In *Vasoactive Intestinal Peptide, Pituitary Adenylate Cyclase-Activating Polypeptide and Related Regulatory Peptides: from Molecular Biology to Clinical Applications*, pp 376–380. Ed G Rosselin. Singapore: World Scientific.

Matsuda K, Takei Y, Katoh JI, Shioda S, Arimura A & Uchiyama M 1997*a* Isolation and structural characterization of pituitary adenylate cyclase-activating polypeptide (PACAP)-like peptide from the brain of the teleost, stargazer *Uranoscopus japonicus*. *Peptides* **18** 725–727.

Matsuda K, Shioda S, Arimura A & Uchiyama M 1997*b* The study of pituitary adenylate cyclase-activating polypeptide (PACAP)-like immunoreactivity in the brain of teleost, stargazer *Uranoscopus japonicus*. *Zoological Science* **14** 645–650.

Matsuda K, Yoshida T, Nagano Y, Kashimoto K, Yatohgo T, Shimomura H, Shioda S, Arimura A & Uchiyama M 1998 Purification and primary structure of pituitary adenylate cyclase-activating polypeptide (PACAP) from the brain of an elasmobranch, stingray *Dasyatis akajei*. *Peptides* **19** 1489–1495.

Mayo KE, Vale W, Rivier J, Rosenfeld MG & Evans RM 1983 Expression-cloning and sequence of a cDNA encoding human growth hormone-releasing factor. *Nature* **306** 86–88.

Mayo KE, Cerelli GM, Lebo RV, Bruce BD, Rosenfeld MG & Evans RM 1985*a* Gene encoding human growth hormone-releasing factor precursor. Structure, sequence, and chromosomal assignment. *PNAS* **82** 63–67.

Mayo KE, Cerelli G, Rosenfeld MG & Evans RM 1985*b* Characterization of cDNA and genomic clones encoding the precursor to rat hypothalamic growth hormone-releasing factor. *Nature* **314** 464–467.

Mayo KE, Godfrey PA, Suhr ST, Kulik DJ & Rahal JO 1995 Growth hormone-releasing hormone: synthesis and signaling. *Recent Progress in Hormone Research* **50** 35–73.

Melamed P, Eliahu N, Levavi-Sivan B, Ofir M, Farchi-Pisanty O, Rentier-Delerue F, Smal J, Yaron Z & Naor Z 1995 Hypothalamic and thyroidal regulation of growth hormone in tilapia. *General and Comparative Endocrinology* **97** 13–30.

Miyata A, Arimura A, Dahl DH, Minamino N, Uehara A, Jiang L, Culler MD & Coy DH 1989 Isolation of a novel 38 residue hypothalamic peptide which stimulates adenylate cyclase in pituitary cells. *Biochemical and Biophysical Research Communications* **164** 567–574.

Miyata A, Dahl DH, Jiang L, Kitada C, Kubo K, Fujino M, Minamino N & Arimura A 1990 Isolation of a neuropeptide corresponding to the N-terminal 27 residues of the pituitary adenylate cyclase activating polypeptide with 38 residues (PACAP38). *Biochemical and Biophysical Research Communications* **170** 643–648.

Montero M, Yon L, Rousseau K, Arimura A, Fournier A, Dufour S & Vaudry H 1998 Distribution, characterization, and growth hormone-releasing activity of pituitary adenylate

cyclase-activating polypeptide in the European eel *Anguilla anguilla*. *Endocrinology* **139** 4300–4310.
Moody AJ, Thim L & Valverde I 1984 The isolation and sequencing of human gastric inhibitory peptide (GIP). *FEBS Letters* **172** 142–148.
Mutt V, Jorpes JE & Magnusson S 1970 Structure of porcine secretin. The amino acid sequence. *European Journal of Biochemistry* **15** 513–519.
Nagy GM, Vigh S & Arimura A 1993 PACAP induces prolactin and growth hormone release in lactating rats separated from their pups. *Endocrine Journal* **1** 169–173.
Nielsen HS, Hannibal J & Fahrenkrug J 1998 Expression of pituitary adenylate cyclase activating polypeptide (PACAP) in the postnatal and adult rat cerebellar cortex. *Neuroreport* **9** 2639–2642.
Ogi K, Kimura C, Onda H, Arimura A & Fujino M 1990 Molecular cloning and characterization of cDNA for the precursor of rat pituitary adenylate cyclase-activating polypeptide (PACAP). *Biochemical and Biophysical Research Communications* **173** 1271–1279.
Ohkubo S, Kimura C, Ogi K, Okazaki K, Hosoya M, Onda H, Miyata A, Arimura A & Fujino M 1992 Primary structure and characterization of the precursor to human pituitary adenylate cyclase-activating polypeptide. *DNA and Cell Biology* **11** 21–30.
Okazaki K, Itoh Y, Ogi K, Ohkubo S & Onda H 1995 Characterization of murine PACAP mRNA. *Peptides* **16** 1295–1299.
Olivereau M, Olivereau J & Vandesande F 1990 Localization of growth hormone-releasing factor-like immunoreactivity in the hypothalamo–hypophysial system of some teleost species. *Cell and Tissue Research* **259** 73–80.
Ono M, Miki N, Demura H, Tadokoro K, Nagafuchi S & Yamada M 1994 Molecular cloning of cDNA encoding the precursor for hamster hypothalamic growth hormone-releasing factor. *DNA Sequence* **5** 93–102.
Parker DB, Coe IR, Dixon GH & Sherwood NM 1993 Two salmon neuropeptides encoded by one brain cDNA are structurally related to members of the glucagon superfamily. *European Journal of Biochemistry* **215** 439–448.
Parker DB, Power ME, Swanson P, Rivier J & Sherwood NM 1997 Exon skipping in the gene encoding pituitary adenylate cyclase-activating polypeptide in salmon alters the expression of two hormones that stimulate growth hormone release. *Endocrinology* **138** 413–423.
Peeters K, Berghman LR & Vandesande F 1998*a* Comparative distribution of pituitary adenylate cyclase-activating polypeptide and vasoactive intestinal polypeptide immunoreactivity in the chicken forebrain. *Annals of the New York Academy of Sciences* **839** 417–419.
Peeters K, Langouche L, Vandesande F, Darras VM & Berghman LR 1998*b* Effects of pituitary adenylate cyclase-activating polypeptide (PACAP) on cAMP formation and growth hormone release from chicken anterior pituitary cells. *Annals of the New York Academy of Sciences* **865** 471–475.
Peng C, Huang YP & Peter RE 1990 Neuropeptide Y stimulates growth hormone and gonadotropin release from the goldfish pituitary *in vitro*. *Neuroendocrinology* **52** 28–34.
Pohl M & Wank SA 1998 Molecular cloning of the helodermin and exendin-4 cDNAs in the lizard. *Journal of Biological Chemistry* **273** 9778–9784.
Rivier J, Spiess J, Thorner M & Vale W 1982 Characterization of growth hormone-releasing factor from a human pancreatic islet tumour. *Nature* **300** 276–278.
Rousseau K, Le Belle N, Marchelidon J & Dufour S 1999 Evidence that corticotropin-releasing hormone (CRH) acts as a growth hormone-releasing factor in a primitive teleost, the European eel *Anguilla anguilla*. *Journal of Neuroendocrinology* **11** 385–392.
Satoh M, Uchiyama M, Nakajo S, Shioda S, Arimura A & Matsuda K 1999 Expression of pituitary adenylate cyclase activating polypeptide (PACAP) mRNA in the brain of a teleost, stargazer. *Zoological Science* **16** (Suppl) 18.
Sawangjaroen K & Curlewis JD 1994 Effects of pituitary adenylate cyclase-activating polypeptide (PACAP) and vasoactive intestinal polypeptide (VIP) on prolactin, luteinizing hormone and growth hormone secretion in the ewe. *Journal of Neuroendocrinology* **5** 549–555.
Sawangjaroen K, Anderson ST & Curlewis JD 1997 Effects of pituitary adenylate cyclase-activating polypeptide (PACAP) and vasoactive intestinal polypeptide (VIP) on hormone secretion from sheep pituitary cells *in vitro*. *Journal of Neuroendocrinology* **9** 279–286.
Spiess J, Rivier J & Vale W 1983 Characterization of rat hypothalamic growth hormone-releasing factor. *Nature* **303** 532–535.
Suhr ST, Rahal JO & Mayo KE 1989 Mouse growth hormone-releasing hormone: precursor structure and expression in brain and placenta. *Molecular Endocrinology* **3** 1693–1700.
Trudeau VL, Somoza GM, Nahorniak CS & Peter RE 1992 Interactions of estradiol with gonadotropin-releasing hormone and thyrotropin-releasing hormone in the control of growth hormone secretion in the goldfish. *Neuroendocrinology* **56** 483–490.
Vaudry D, Gonzalez BJ, Basille M, Yon L, Fournier A & Vaudry H 2000 Pituitary adenylate cyclase-activating polypeptide and its receptors: from structure to functions. *Pharmacological Reviews* **52** 269–324.
Vaughan JM, Rivier JE, Spiess J, Peng C, Chang JP, Peter P & Vale W 1992 Isolation and characterization of hypothalamic growth hormone-releasing hormone from common carp *Cyprinus carpio*. *Neuroendocrinology* **56** 539–549.
Velkeniers B, Zheng L, Kazemzadeh M, Robberecht P, Vanhaelst L & Hooghe-Peters EL 1994 Effect of pituitary adenylate cyclase-activating polypeptide 38 on growth hormone and prolactin expression. *Journal of Endocrinology* **143** 1–11.
Wei L, Chan WWS, Butler B & Cheng K 1993 Pituitary adenylate cyclase-activating polypeptide-induced desensitization on growth hormone release from rat primary pituitary cells. *Biochemical and Biophysical Research Communications* **197** 1396–1401.
Wong AOL, Leung MY, Shea WLC, Tse LY, Chang JP & Chow BKC 1998 Hypophysiotropic action of pituitary adenylate cyclase-activating polypeptide (PACAP) in the goldfish: immunohistochemical demonstration of PACAP in the pituitary, PACAP stimulation of growth hormone release from pituitary cells, and molecular cloning of pituitary type I PACAP receptor. *Endocrinology* **139** 3465–3479.
Yamamoto K, Hashimoto H, Hagihara N, Nishino A, Fujita T, Matsuda T & Baba A 1998 Cloning and characterization of the mouse pituitary adenylate cyclase-activating polypeptide (PACAP) gene. *Gene* **211** 63–69.
Yon L, Feuilloley M, Chartrel N, Arimura A, Conlon JM, Fournier A & Vaudry H 1992 Immunohistochemical distribution and biological activity of pituitary adenylate cyclase-activating polypeptide (PACAP) in the central nervous system of the frog *Rana ridibunda*. *Journal of Comparative Neurology* **324** 485–499.
Yon L, Jeandel L, Chartrel N, Feuilloley M, Conlon JM, Arimura A, Fournier A & Vaudry H 1993 Neuroanatomical and physiological evidence for the involvement of pituitary

adenylate cyclase-activating polypeptide in the regulation of the distal lobe of the frog pituitary. *Journal of Neuroendocrinology* **5** 289–296.

Yoshida T, Itoh Y, Uchiyama M, Shioda S, Arimura A, Kashimoto K & Matsuda K 1999 Distribution of pituitary adenylate cyclase activating polypeptide (PACAP)-like immunoreactivity in the brain of an elasmobranch, stingray. *Zoological Science* **16** (Suppl) 18.

RECEIVED 2 March 2000